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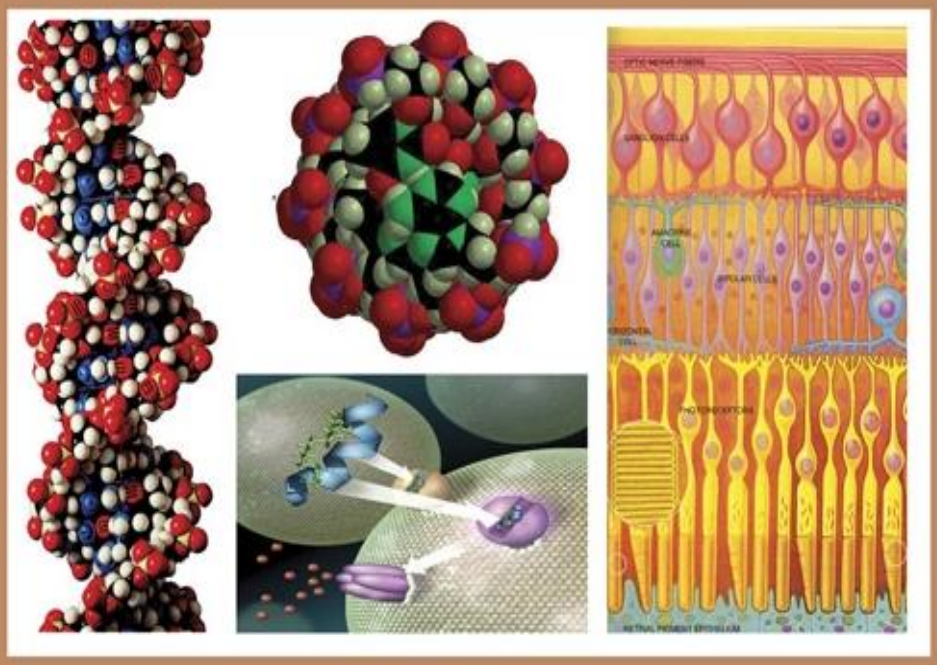

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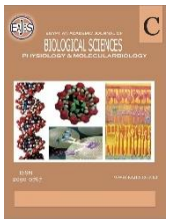

Phytochemical Screening and Antioxidant Properties of *Nerium oleander* Growing in Algeria

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ABSTRACT

Plant species of medicinal interest are involved in various sectors in their raw state or in the form of oils, extracts, aqueous or organic solutions. Their preparations contain one or more active ingredients that can be used for therapeutic purposes. Medicinal plants are recognized for physicochemical properties and richness in natural drugs belonging to different molecule classes such as terpenoids and phenolic compounds, like flavonoids, tannins, coumarins and phenolic acids that contribute to effective protection against numerous pathological processes. This study was devoted to phytochemical screening and evaluation of biological properties, namely, the antioxidant activities of hydroethanolic and infusion extracts prepared from Algerian Sahara *Nerium oleander*. Phytochemical tests applied to the studied plant showed the presence of several families of chemical compounds, including tannins and flavonoids. All samples exhibited interesting antioxidant activity when compared to the standard Trolox, but the hydroethanolic extract (EC_{50} values between 18 and 75 µg/mL) presented the highest bioactivity. The contemporary presence of bioactivities suggests that the Saharan *Nerium oleander* may be a source for such new preservatives in the food and pharmaceutical industries.

INTRODUCTION

In Africa, where herbal medicines are still used by many people for health care, the therapeutic power of plants was known empirically. Plants were known empirically. The Algerian flora, with its different species belonging to several botanical families, remains very little explored both in phytochemical and pharmacological terms. The abundance of active principles gives the plant remarkable pharmacological properties, which could justify its multiple therapeutic indications and the way it is used in radiotherapy (Lamia. S. *et al.*, 2022).

This is why, much attention has been paid to their extraction and isolation from herbs to replace synthetic preservatives in food products in order to prevent food and living systems from peroxidative damage (Takwa *et al.*, 2018).

Oleander should not be confused with Laurel. When we speak of the bay in flower, we are in fact talking about oleander. This is not used in cooking because it is poisonous. Many other plants are also called laurel, but they do not belong to the Laurus genus or even, for the most part, to the Lauraceae family. These include the following:

- the oleander (*Nerium oleander*), from the Apocynaceae family, which is extremely poisonous.
- laurel (*Viburnum tinus*), from the Caprifoliaceae family
- the Portuguese laurel (*Prunus lusitanica*) which is rosacea.

Of all the bay trees, only the oleander is edible. A mix-up could have serious consequences: using the leaves of other "laurels" in cooking or in infusions could lead to poisoning. Of all the laurels, the oleander is one of the most dangerous plants, with all its parts being toxic. The ingestion of a single leaf can be fatal for an adult, due to the heart problems often caused. This perennial tree has elegant, fragrant flowers, which are not necessarily pink, some of which are white, pinkish-red, orange, or orange-red.

MATERIALS AND METHODS

1. Plant Material:

The plant was collected in the Wilaya of El Bayadh in October 2021(Fig. 1). The Wilaya of El Bayadh being an integral part of the high steppe plains region of southwest Algeria is located at 1313m altitude, 33°40′ 49″ North and 1° 1′ 13″ East.

We used the complete plant which will be kept in a hermetically sealed glass jar to preserve its initial quality.

Fig. 1: *Nerium oleander*

2. Extraction Procedure:

Hydroethanolic and infusion extracts were prepared from our plant. The hydroethanolic extraction (80% ethanol, 30 mL) was performed by maceration (150 rpm), with 1 g of each sample at 25°C for 1 hr and then filtered; the residue was re-extracted, using the same methodology. Afterwards, the extracts were evaporated in order to remove the ethanol, under reduced pressure. For aqueous extracts, 2 g of plant material was infused with boiling distilled water for 15 min and then filtered. Both extracts were previously frozen before

lyophilization, in order to obtain a dry extract.

The lyophilized hydroethanolic and infusion extracts were dissolved in ethanol/water (80:20, v/v) and water, respectively, to obtain a stock solution of 10 mg/mL for the antioxidant activity assays; 20 mg/mL in culture medium for the antimicrobial assays; and, finally, 8 mg/mL in water for anti-inflammatory and cytotoxicity tests. In the bioactivity evaluation assays, the stock solutions were further diluted and tested.

3. Phytochimical Screening:

To get an idea about the main families that can be found in plant material, we have made a phytochemical screening. This one is either based on the formation of insoluble or colored complexes. The observed coloration is usually due to the formation of conjugation or instauration in a molecule. In such tests of characterization, we cause this instauration using a suitable reagent. We have characterized the different chemical groups (tannin, flavonoid, Sterols and steroids, alkaloids and saponins) by referring to the techniques described by (Paris *et al.,* 1969; Trease and Evans, 1996) with some modifications.

4. Antioxidant Activity Assays*:*

Four different *in vitro* methods were used to evaluate the antioxidant activity, DPPH radical-scavenging activity, reducing power, β-carotene bleaching inhibition assay and lipid peroxidation inhibition by TBARS using methodologies previously described by Sobral *et al.* (2016). Results were expressed as EC50 value (µg/mL) and Trolox was used as a positive control.

RESULTS AND DISCUSSION

1. Phytochimical Composition;

As shown in Table 1, Phytochemical analysis demonstrated the presence of common phytoconstituents like sterols and steroids, tannins, saponins, flavonoids, triterpenic heterisides and the absence of alkaloids, coumarins and anthocyanin (Table 1). Our results are consistent with those obtained by Makloufi *et al.* (2012).

Table 1: Phytochemical analysis of *Nerium oleander* (Del).

Compounds	*Nerium oleander* (Del)
Starch	+
Saponins	+
Tanin cathéchique	--
Tannin gallique	+
Flavonoid	++
reducing Compounds	-
Alkaloïd	-
coumarins	-
Anthocyanin	+
Sterols and steroids	+
Steroid heterosides	+
Triterpenic heteroside	++

(-) Abscens, (+) presence, (++) strongly present.

Nerium oleander shows richness in phenolic compounds namely, tannins, flavonoids and saponins, these molecules have analgesic, anti-inflammatory and anti-edematous properties (Roux *et al.,* 2007). This diversity of compounds could justify their use in traditional treatments for venous insufficiency, functional signs of hemorrhoidal crisis and disorders of capillary fragility.

2. Antioxidant Activity:

The results regarding the antioxidant activity of both *Nerium oleander* (Del) hydroethanolic and infusion extracts are shown in Table 2. In the present investigation the antioxidant activities were determined using four assays were carried out: DPPH radical scavenging activity, reducing power, β-carotene bleaching inhibition and lipid peroxidation inhibition in brain cell homogenates (TBARS). The results were compared with the standard Trolox and expressed as EC_{50} values. Based on these results, *Nerium oleander* extracts showed interesting antioxidant properties, particularly for hydroethanolic extract and the TBARS assay, in which the samples demonstrated to be two to three times more effective than Trolox, with EC_{50} of 26.8µg/mL.

Table 2. Antioxidant activity and NO production inhibition capacity of *Nerium oleander* extracts.

Antioxidant activity (EC_{50}values, µg/mL)	***Nerium oleander***	
	Hydroethanolic extract	Infusion extract
DPPH scavenging activity	61.2	90.13
Reducing power	48.24	71.9
β-carotene bleaching inhibition	21	44.5
TBARS inhibition	23,06	45.
Anti-inflammatory activity (EC_{50} values, µg/mL)		
Nitric oxide (NO) production	327	>400

*Trolox and Dexamethason for antioxidant and anti-inflammatory activities, respectively.

The antioxidant activity was expressed as EC_{50} values, which means that higher values correspond to lower reducing power or antioxidant potential. EC_{50}: extract concentration corresponding to 50% of the antioxidant activity or 0.5 of absorbance in reducing power assay. Results of anti-inflammatory activity are expressed in EC_{50} values: sample concentration providing 50% of inhibition of nitric oxide (NO) production.

The Nerium species, Nerium indicum and Nerium oleander are toxic plants. As such their use in herbal medicine is limited to external use. Neriumindicum is widely used in traditional Chinese medicine to stimulate the heart muscles and relieve pain and as an insecticide (EunJeong. S., 2001; Shan Yu. M. *et al.,* 2004). Bark of N. indicum has been reported to have molluscicidal activity against Lymnaeaacuminata (Singh. S. *et al.,* 1998). Despite its established toxicity, N. oleander species are used in traditional medicine for the treatment of many diseases and are in fact included in several folk pharmacopoeias (Adom. R. O. *et al.,* 2003).

Nerium oleander is used in traditional medicine for the treatment of many diseases and is included in several local pharmacopoeias (Adom. R. O. *et al.,* 2003; Almahy. H. A. *et al.,* 2006).

Nerium oleander extracts showed anti-lipid peroxidation proprieties, which has a great interest in food preservation (Takwa *et al.,* 2018). The ability of plant extracts to scavenge DPPH radical assay showed that Hydroethanolic extract presented low EC_{50} values compared to infusion with (75.2 and 94.14µg/mL, respectively), The results obtained were less than those reported by Metrouh Amir *et al.* (2015), who have evaluated solvent effect on total phenolic contents and antioxidant activities of *Nerium oleander* from Algerian septentrional sahara and showed an EC_{50}(4.38 and 4.14 µg/mL) for aqueous and ethanol (50%) extracts, respectively. Moreover, the activity of all samples was weak than the one displayed by the commercial standard Trolox (EC_{50}=42±1µg/mL). In fact, both extracts from the studied plant revealed reducing power, which increased with the increase in the extract concentration, ranging from EC_{50} (56.24 and 89.8 µg/mL) for Hydroethanolic and infusion extracts respectively, and being less than Trolox (41±1 µg/mL). Once more, *Nerium oleander* hydroethanolic and infusion extracts exhibited a weak ability to reduce Fe^{3+} to Fe^{2+} compared to those reported by Metrouh Amir *et al.* (2015). The free radical scavenging activity of *Nerium oleander* was also determined using β-carotene bleaching inhibition assay. For this methodology, the hydroethanolic extract demonstrated the highest activity, which is similar to standard Trolox (EC_{50} values of 18 µg/mL), however, the infusion extract showed an activity (EC_{50} values of 41,5 µg/mL). Also, TBARS inhibition confirmed, the best scavenging activity of *Nerium oleander* hydroethanolic extract (EC_{50} values of 42 µg/Ml). The effectiveness of the hydroethanolic extracts can be explained by their higher concentration of phenolic compounds, which could influence their capacity to

scavenge free radicals and prevent lipid peroxidation (Gardi *et al.*, 2015).

Nerium oleander is a plant that is toxic if its various parts are ingested (leaves, flowers, stems, etc.). Its toxicity to humans, animals and certain insects has been the subject of several studies (Adom. R. O. *et al.*, 2003; Almahy. H. A. *et al.*, 2006; Barbosa. R. R. *et al.*, 2008).

Nerium oleanderis more often associated with accidental intoxication in children or even in domestic animals (Bruneton. J., 2001). However, suicide attempts with *Nerium oleander* are regularly reported by toxicologists in different parts of the world, and a case of criminal use has recently been reported (Bourgeois. B. *et al.*, 2005).

Poisoning can be caused by the ingestion of a single green or dried leaf, which can be fatal to an adult. The first signs of poisoning are unconsciousness, irritation of mucous membranes, nausea, vomiting, abdominal pain, diarrhoea, polypnoea, severe cardiac disorders, and skin burns sometimes reported in susceptible individuals. Symptoms appear several hours (72 h) after ingestion of a toxic quantity (Adom. R. O. *et al.*, 2003).

The cardiotonic heterosides, which are the main constituents of N. oleander, are the recognised toxicants of this species (Bruneton. J., 2001).

Conclusion

In this study the extract of the plant Nerium oleander was obtained by decoction maceration, in this work, we evaluated the antioxidant activity.

The results obtained in this study are interesting, but further studies are needed to understand the molecular and cellular mechanisms of these effects. These studies should also be oriented towards the determination of the active compounds in the N.O U.D extracts and the evaluation of their effects on the mechanisms of this activity.

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